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ANTIMICROBIAL ACTIVITY OF PROBIOTICS FROM MOTHER MILK, CURD AND YOGHURT SAMPLES

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ABSTRACT

Important microorganisms as a probiotics were obtained from Mother Milk, Curd and Yoghurt sample after enrichment in MRS medium. Isolates were further tested for their antimicrobial activity against *E.coli*, *E.aerogen*, *S.typhi* B, *S.aureus*, *B.cereus*, *B.megaterium*, *B.subtilis*, *Pseudomonas* using N-agar plates by well –diffusion assay. Isolates have potent antimicrobial activities against common laboratory isolates. Isolates were found to inhibit growth of most of isolates tested, if not all.

Key words: probiotics, Mother Milk, Curd, Yoghurt, antimicrobial activity

INTRODUCTION

Probiotics are living, health - promoting micro organisms that are incorporated into various kinds of foods. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the bactericidal activity of the bile salts, as well as the production of lactic acid that inhibits the growth of other microorganisms, allow them to be established in the intestinal tract (Catanzaro and Green, 1997). The reported health benefits include: boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from various causes, prevention of cancer, reduction of the risk of inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins, and detoxification and protection from toxins (Hobbs, 2000).

The concept of probiotics progressed around 1900, when Elie Metchnikoff hypothesized that the long

and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk and milk products (Kopp-Hoolihan, 2001). Members of the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are the most common probiotics used in commercial fermented and non-fermented dairy products today (Heller, 2001). Among the popular probiotic foods in the Philippine market today are some infants' milk, fermented milk drinks and yogurts.

This study was conducted to determine the presence of antimicrobial activities among the probiotics incorporated into these different food products against common microbial pathogens. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public.

MATERIALS AND METHODS

Isolation

Probiotic isolates were isolated from Mother Milk, Curd and Yoghurt sample. Three groups of isolates

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were tested. The first group was comprised of Mother Milk. The second group was made-up of curd products. The last group included Yoghurt as a sample. The samples were enriched in De Man Rogosa Sharpe (MRS) medium MRS Broth and after the enrichment, enriched culture were streaked on MRS agar plate. The isolates were subsequently grown and maintained in the MRS agar media, which proved to be the most suitable for the growth of each isolate. The plates were incubated at 37°C for 48 hours. The isolated organisms were Gram stained for the study of microscopic morphology. Stock cultures of the probiotics were maintained in their respective culture media at 4°C.

Culture of Test Microbial Isolates

The test microbial isolates were comprised of Gram negative bacteria *Escherichia coli*, *E.aerogen*, *S.typhi B*, *Pseudomonas*, Gram positive bacteria *S.aureus*, *B.cereus*, *B.megaterium*, *B.subtilis*. Organisms were maintained in Nutrient Agar slants and tubes kept at 4°C.

Determination of Antimicrobial Activities

Isolates were inoculated in MRS broth and incubated at 37° c for 24hrs for activation of cultures and then centrifuged at 3000 rpm for 15min. and supernatant was collected to study antibacterial activity. The test organisms from the stock cultures were subcultured in Nutrient broth under aerobic condition at 37° C for 18 hrs. 0.2ml of activated test cultures was inoculated in molten agar and poured in sterile plates than allowed to solidify. Wells were prepared at equal distance in solidified agar plates using cup-borer. Supernatant of isolates was poured in respective plates. The plates were incubated at 37°c for 24hrs to observe zone inhibition. Zone of diameter was measured and then with the help of that zone index was calculated.

RESULTS AND DISCUSSION

Isolation

From the Mother Milk sample, Milk, 3 and 1C, from Curd Sample Pc, Cub, Cuc, and Cue and from yoghurt sample Yg1, Yg2 and Yg3 total 10 isolates were obtained.

Microscopic Characterization

All isolates were Gram Positive. Out of 10 Isolates 8 isolates were cocci, 2 isolates were rod in shaped (Figure-1 and 2).

Determination of antimicrobial activities of isolated probiotics

Results showed that all the isolates from the different samples were able to inhibit the growth of some, if not all, of the test microbial isolates. The spectrum of their antimicrobial activity varied.

Photograph: 1-Inhibition of *Pseudomonas* by Milk Isolates.

Photograph:2-Inhibition of *B.megaterium* by Curd Isolates. Photograph:3-Inhibition of *B.megaterium* by Yoghurt Isolates.

Results of the study showed the antimicrobial activity of the isolates. This may be due to the production of bacteriocins that acted as antibiotic agents (Bezkorovainy, 2001). Other than bacteriocins, some are also capable of reuterine production that is known to act as an antibacterial compound (Marteau *et al.*, 1990).

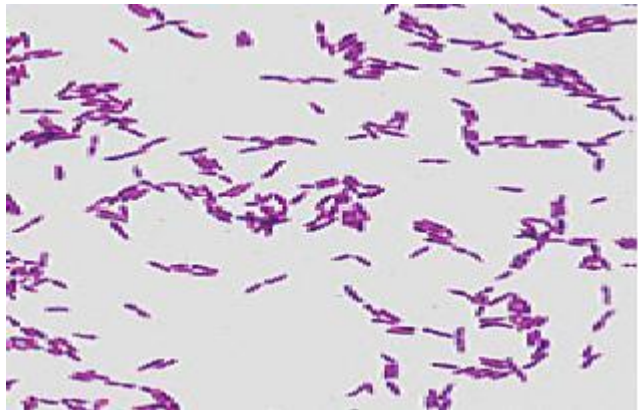
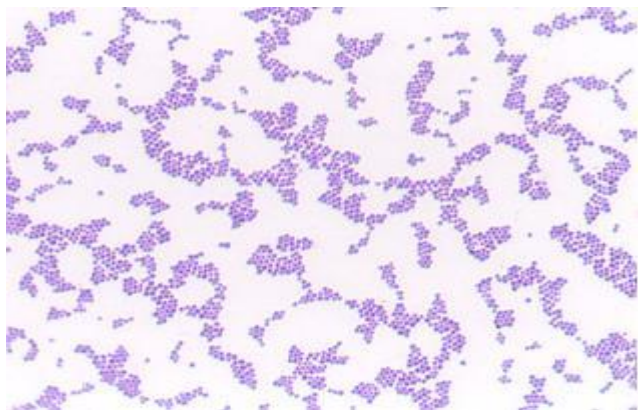
Graph 1, 2 and 3 shows that milk sample isolates, curd sample isolate as well as yoghurt sample isolates indicates antibacterial activity against each organism.

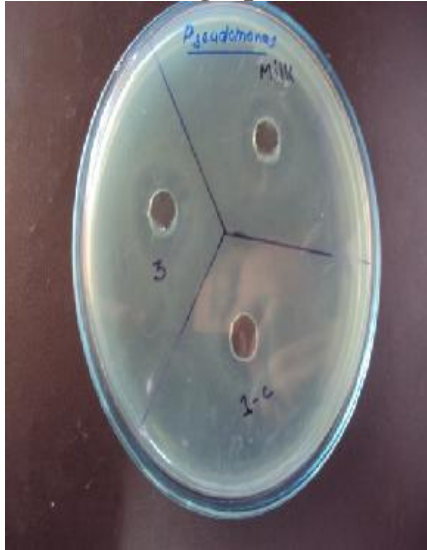
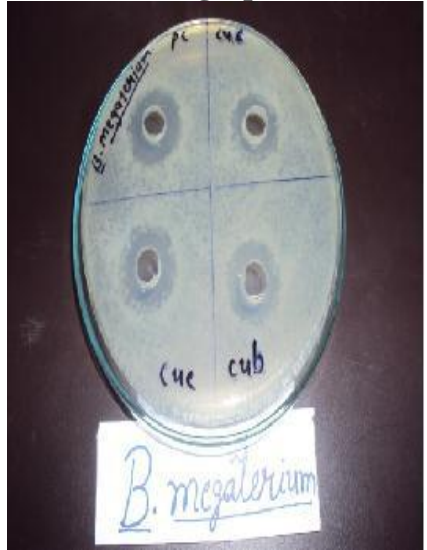
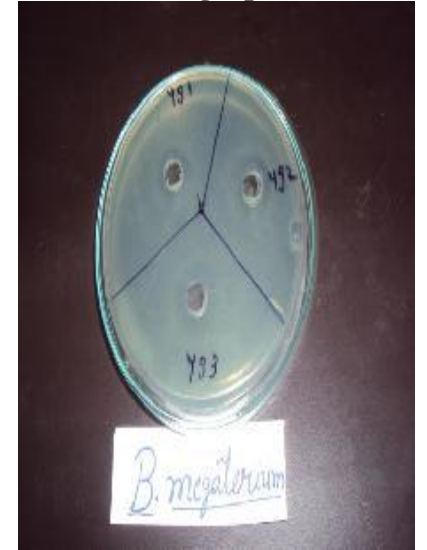
Graph1 indicates that each isolates shows comparative inhibition pattern and from graph it can be observed that 1-C gives highest antimicrobial activity against *S.aureus*. While 3 gives maximum zone of inhibition against *Pseudomonas* and *B.cereus*. From this it can be summarized that isolate 1-C can be more useful for treating and preventing respiratory infections. *Lactobacillus* spp. has demonstrated inhibition of growth of human pathogens due to acid production (Servin, 2004). Milk isolates cannot inhibit *B.subtilis* and 1c both were unable to inhibit *B.cereus*.

Graph 2 indicates that all isolates have antimicrobial activity against *E.coli*, *E.aerogens*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Salmonella*, *Shigella*, *S.aureus* and *Pseudomonas* species. *S.aureus* was more inhibited by PC, while contrastingly PC was unable to inhibit *B.subtilis*. Cub & Cuc were more inhibitory to *E.aerogen*. Cuc has given maximum zone of inhibition against *S.typhi B*. Thus it can be interpreted that isolates. Cuc can be used as food supplementary in the case of Typhoid infection.

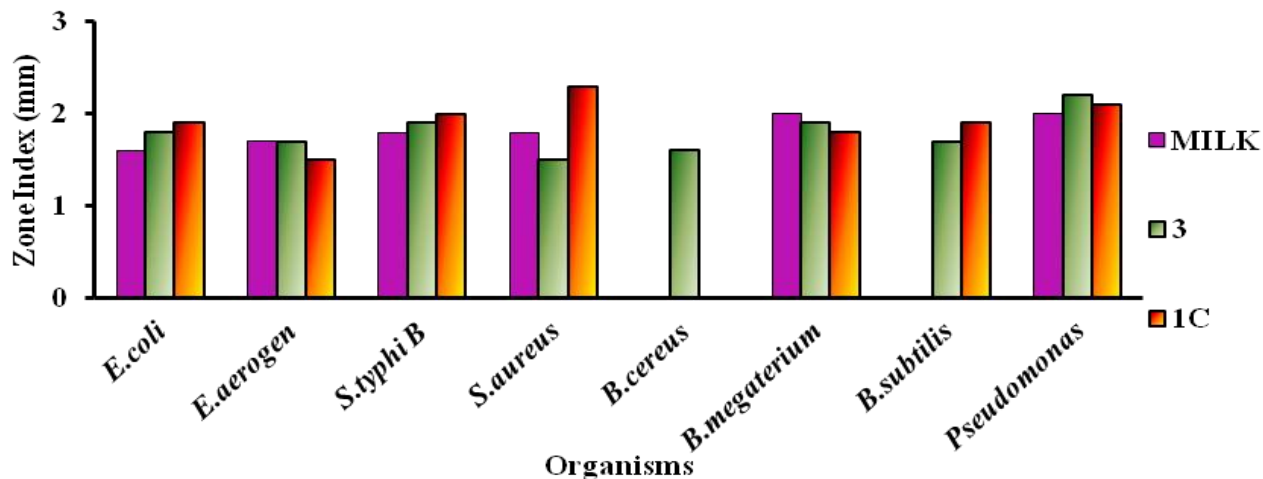
Graph 3 shows that Yg₁ gives highest antibacterial activity against *S.typhi para B*. While Yg₂ gives maximum zone of inhibition against *B.megaterium*. This could be the reason for the beneficiary action of Yakult, Yoghurt and curd. The result of the study indicates that probiotics are helpful in the protection and improvement of our intestinal flora. Consuming these products can help protect one from occurrences of diarrhea, food poisoning and even systemic and enteric infections. The verified antimicrobial activity of the probiotics supports the development of these functional foods as a key to the improvement of the health of the consuming public.

This study was conducted to determine the presence of antimicrobial activities among the probiotics incorporated into these different food products against common microbial pathogens. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public.

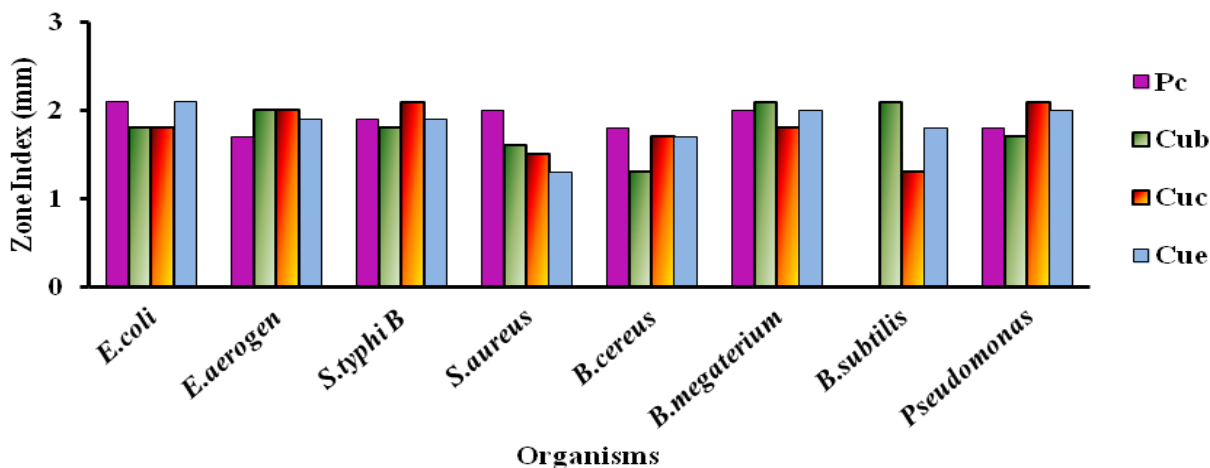
<p>Figure-1 Morphology of isolates natural samples</p> 	<p>Rod</p> <p>Milk, Yg2,</p>
<p>Figure-2 Morphology of isolates from natural samples</p> 	<p>Cocci</p> <p>3,1C Pc, Cub, Cuc, Cue, Yg1, Yg3</p>

<p>Photograph: 1</p> 	<p>Photograph: 2</p> 	<p>Photograph: 3</p> 
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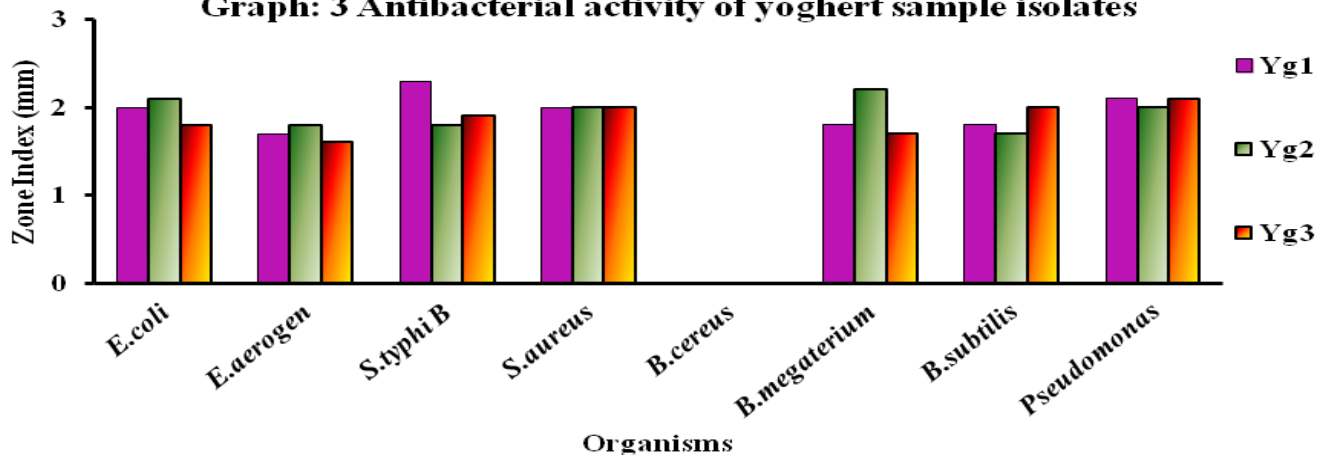
Graph: 1 Antibacterial activity of milk sample isolates



Graph: 2 Antibacterial activity of curd sample isolates



Graph: 3 Antibacterial activity of yoghurt sample isolates



CONCLUSION

The capability of the isolates of Mother Milk, Curd and Yogurt to inhibit the growth or even to kill certain organisms confirms the health benefits that one derives from the consumption of these products. The results of the study suggest that probiotics are helpful in the protection and improvement of our intestinal flora.

Consuming these products can help protect one from occurrences of diarrhoea, food poisoning and even systemic and enteric infections (Marteau *et al.*, 1990; Mc Donagh *et al.*, 1999). The verified antimicrobial activity of the probiotics supports the development of these functional foods as a key to the improvement of the health of the consumers.

REFERENCES

- Bezkorovainy A. Probiotics: Determinants of survival and growth in the gut. *American Journal of Clinical Nutrition*. 2001; 73: 399-405.
- Catanzaro J, Green L. Microbial ecology and probiotics in human medicine (Part II). *Alternative Medicine Review*. 1997; 2: 296-305.
- Heller K. Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *American Journal of Clinical Nutrition*. 2001; 73: 374S-379S.
- Hobbs C. Pro-life therapy with probiotics. Health World Online TM. 2000. <http://www.healthy.net/asp/templates/article.asp?id=953>.
- Kopp-Hoolihan L. Prophylactic and therapeutic use of probiotics: A review. *Journal of American Dietetic Association*. 2001; 101: 229-241.
- Marteau P, Pochart P, Flourie B, *et al.* Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora. *American Journal of Clinical Nutrition*. 1990; 52: 685-88.
- Mc Donagh D, Donnelly WJ, Lawless F, *et al.* Milk and dairy products for better human health. In. Proceedings of the National Dairy Conference, Adare, Co. Limerick, 1999. pp. 51-58.
- Servin AL. Antagonistic activities of *lactobacilli* and *bifidobacteria* against microbial pathogens. *FEMS Microbiology Reviews*. 2004; 28: 405-440.